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# Stress and Vascular Responses: Mitogen-Activated Protein Kinases and Activator Protein-1 as Promising Therapeutic Targets of Vascular Remodeling

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**Abstract.** Mitogen-activated protein kinases (MAP kinases), including extracellular signal-regulated kinase (ERK), c-Jun $NH_2$-terminal kinase (JNK), and p38, play a central role in cellular responses by various stress stimuli such as cell proliferation, apoptosis, migration, or gene expression. Furthermore, activator protein-1 (AP-1), a transcription factor which can be activated by MAP kinases, also is involved in a variety of celllar responses, as well as MAP kinases. MAP kinases and AP-1 are significantly activated in vascular tissues by hypertension, angiotensin II, or balloon injury. We have made dominant negative mutants of MAP kinases or c-Jun, to specifically inhibit in vivo activation of MAP kinases or AP-1. Vascular gene transfer of each dominant negative mutant of MAP kinases or c-Jun prevents intimal hyperplasia after balloon injury, which is associated with the inhibition of smooth muscle cell proliferation in the intima and the media and probably also associated with inhibition of smooth muscle cell migration. However, in vitro findings on cultured vascular smooth muscle cells suggest that the molecular mechanism underlying inhibition of intimal hyperplasia may be different among each dominant negative mutant of MAP kinases and c-Jun. MAP kinases and c-Jun seem to be the promising therapeutic target for vascular remodeling.

***Keywords***: gene transfer, mitogen-activated protein kinase, activator protein-1, vascular remodeling

## Introduction

Extracellular stimuli, including mechanical stretch, G protein-coupled receptor agonists, growth factors, cytokines, stresses, etc., lead to a wide variety of cellular responses such as cellular phenotypic change, growth, apoptosis, migration, or gene expressions. The activation of intracellular signal transduction pathways, particularly the activation of protein kinases, is the first important molecular event underlying cellular responses. Mitogen-activated protein kinases (MAP kinases), composed of extracellular signal-regulated kinases (ERKs), c-jun $NH_2$-terminal kinases (JNKs), and p38-MAP kinases, are protein serine/threonine kinases and play a critical role in cell differentiation, growth and apoptosis, and the regulation of various transcription factors and gene expressions (Fig. 1) (1).

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Activator protein-1 (AP-1), one of the main transcription factors activated by either ERK or JNK, play a central

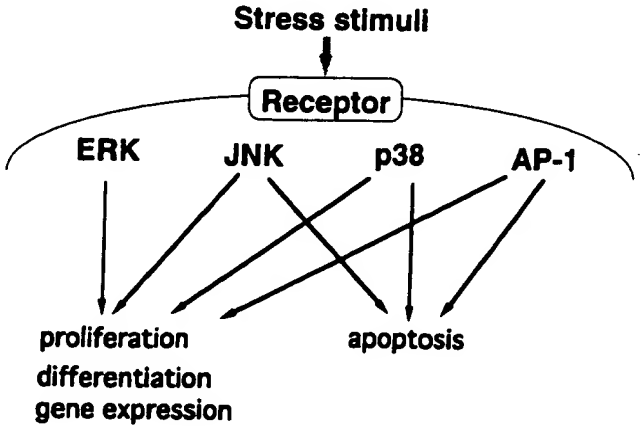


**Fig. 1.** A variety of function of MAP kinases and AP-1. MAP kinases, including ERK, JNK and p38, and AP-1 are activated by various stress stimuli and are responsible for cellular responses.

role in a variety of cellular responses as well as MAP kinases (2, 3). In a series of experiments on rats, we have reported that MAP kinases and AP-1 are significantly activated in hypertrophied heart, balloon-injured artery, and hypertensive vascular or renal tissue (4 – 10). Therefore, it is assumed that MAP kinases may be involved in cardiovascular and renal diseases.

In this review, we will describe our recent work on vascular gene transfer of dominant negative MAP kinases or c-Jun in the balloon injury model and discuss the possible target of MAP kinases and AP-1 for treatment of vascular remodeling.

**Inhibition of activation of MAP kinases by gene transfer of their dominant negative mutants**

Vascular remodeling is characterized by an active process of structural changes involving cellular responses such as cell growth, cell death, cell migration, and accumulation or degradation of extracellular matrix; and it plays a key role in the pathophysiology of various vascular diseases. Arterial balloon injury causes vascular intimal thickening characterized by smooth muscle cell migration and proliferation and the accumulation of extracellular matrix; and it is one of the most popular in vivo models of vascular remodeling. Balloon injury of rat carotid artery induces a rapid and transient activation of JNK and ERK in injured arterial tissue (Fig. 2A). Therefore, we have examined the role of these kinase activations in neointimal formation, by using the in vivo gene transfer technique. We have made the dominant-negative mutants of ERK (DN-ERK) and JNK (DN-JNK) (Fig. 2B). Two days before balloon injury, these mutants were transfected into rat carotid artery, using the hemagglutinating virus of the Japan-liposome method, to specifically inhibit these kinase activations by balloon injury (11). Neointimal formation at 14 or 28 days after injury is prevented by gene transfer of

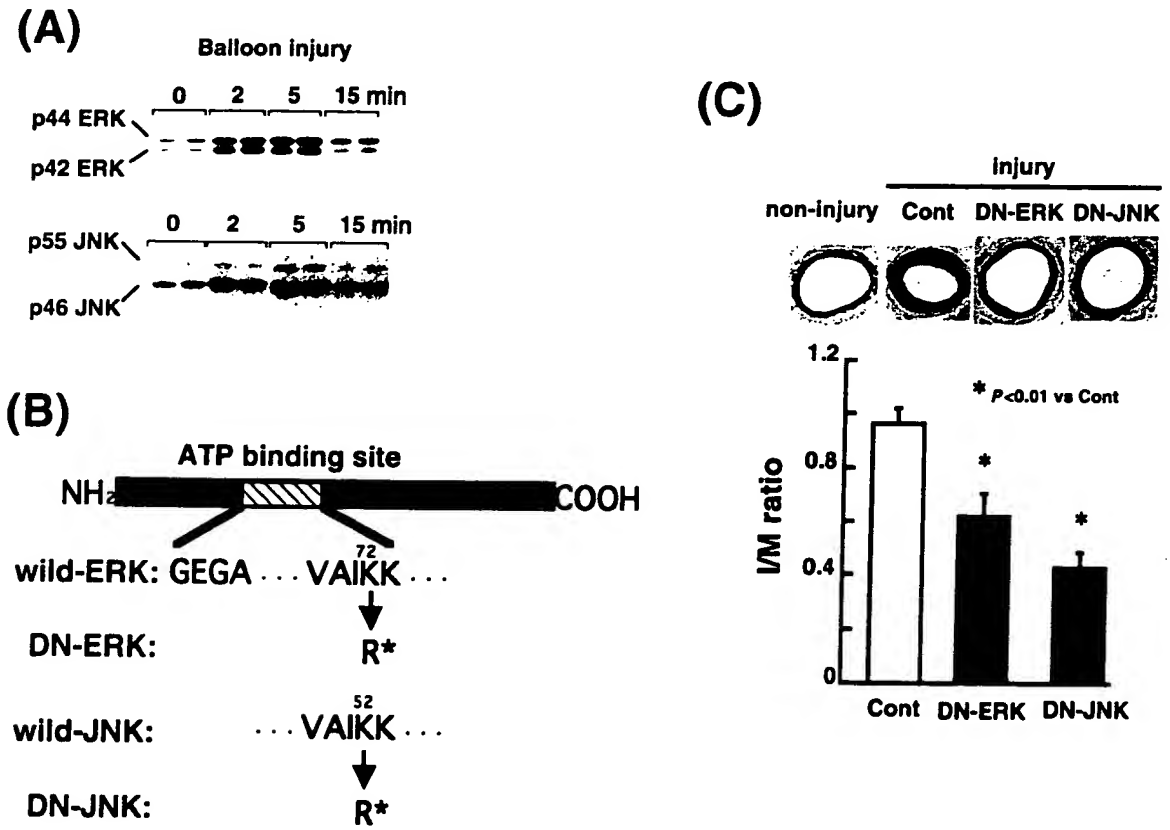


Fig. 2. Inhibition of intimal thickening after balloon injury by gene transfer of dominant negative mutant of ERK and JNK. A: Rat arterial ERK and JNK are rapidly and transiently activated in the injured artery after balloon injury, as shown by the in-gel kinase assay. B: Dominant-negative mutants of p44 ERK cDNA (DN-ERK) and of p46 JNK cDNA (DN-JNK) are produced by the polymerase chain reaction using primers designed to produce a lysine (AAG) – arginine (CGG) substitution at lysine 72 in the ATP binding site of wild-ERK and a lysine (AAG) – arginine (CGG) substitution at lysine 52 in the ATP binding site of wild-JNK, respectively. C: Gene transfer of DN-ERK and DN-JNK into the arterial wall with the HVJ (hemagglutinating virus of the Japan)-liposome prevents intimal hyperplasia 14 days after balloon injury. I/M ratio, ratio of intimal to medial areas.

DN-ERK or DN-JNK (Fig. 2C), which is due to the inhibition of smooth muscle cell proliferation in the intima and the media and is probably also mediated by the inhibtion of smooth muscle cell migration, because infection of cultured smooth muscle cells with recombinant adenovirus containing DN-ERK or DN-JNK prevents smooth muscle cell migration (Y. Zhan et al., submitted). Thus, ERK and JNK activation triggers a series of molecular events leading to neointimal hyperplasia in vivo, thereby supporting the proposal that ERK and JNK may be the new therapeutic targets for prevention of vascular remodeling.

Besides ERK and JNK, p38 is another MAP kinase and it has different downstream cascades from ERK and JNK. We have prepared recombinant adenovirus containing the dominant-negative mutant of p38 cDNA (DN-p38) by the polymerase chain reaction using primers to produce a mutant in the site of dual-activating phosphorylation through substitution of TGY (threonine180-glycine-tyrosine182) with AGF (alanine180-glycine-phenylalanine182). We have found that this adenoviral infection of arterial tissue leads to the inhibition of intimal hyperplasia induced by balloon injury (Y. Zhan et al., submitted). Thus, p38 seems to be a useful therapeutic target for prevention of vascular remodeling, as well as ERK and JNK. However, ERK, JNK, and p38 have differential roles in the pathophysiology of vascular remodeling regarding the molecular mechanism of smooth muscle cell proliferation and the role in gene expression of factors such as transforming growth factor-$\beta$1, plasminogen activator inhibitor-1, and monocyte chemoattractant protein-1 (Y. Zhan et al., submitted). Therefore, we propose that combined gene transfer of DN-ERK, DN-JNK, and DN-p38 may be a more potent therapeutic strategy than single gene transfer.

**Inhibition of AP-1 activation by gene transfer of DN-c-Jun**

As reviewed, c-Jun exerts diverse biological function

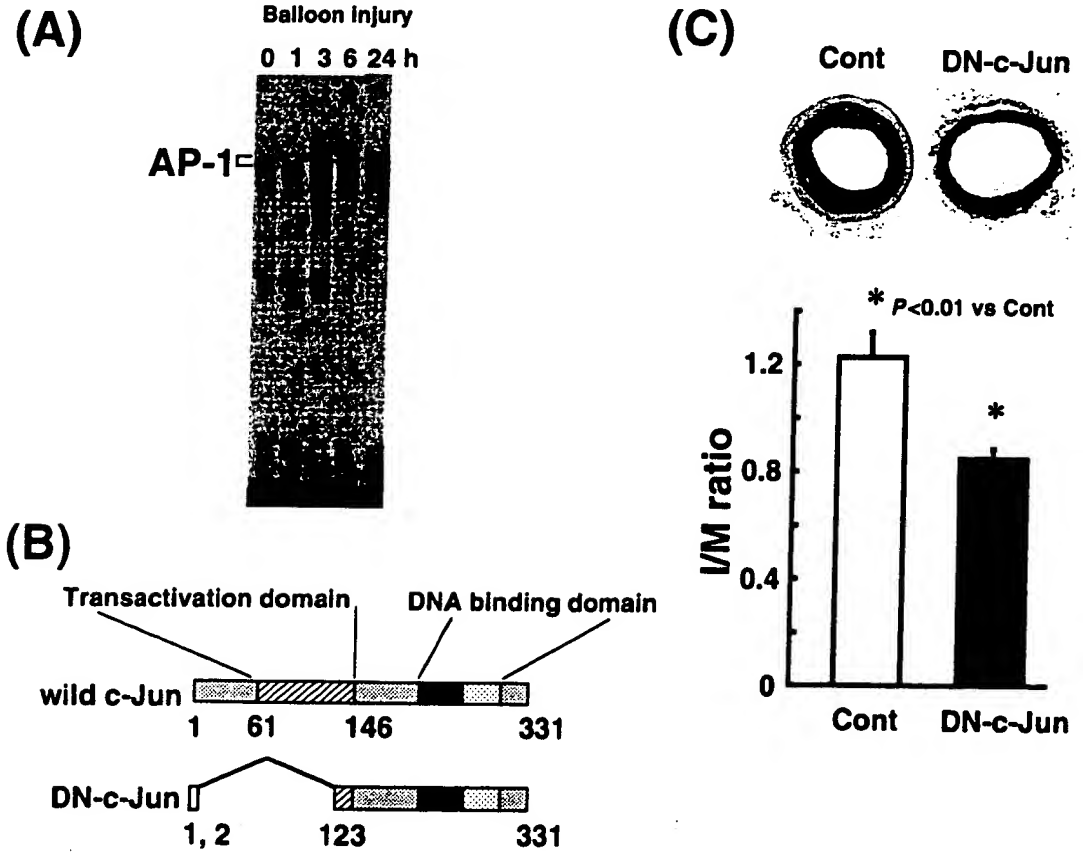


Fig. 3. Inhibition of intimal thickening after balloon injury by gene transfer of dominant negative mutant of c-Jun. A: Arterial AP-1 is activated after balloon injury with a peak at 3 h, as shown by gel mobility shift analysis. B: The dominant negative mutant of c-Jun (DN-c-Jun) is generated by deleting the transactivational domain (amino acids 3 to 122) of wild c-Jun with the polymerase chain reaction. C: Gene transfer of DN-c-Jun prevents arterial intimal hyperplasia 14 days after balloon injury. I/M ratio, ratio of intimal to medial areas.

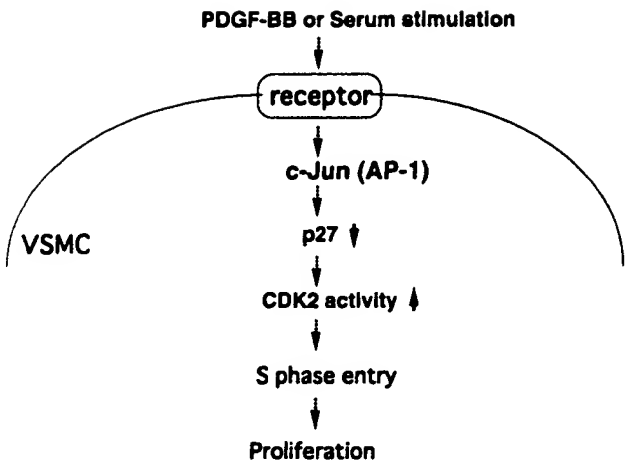


Fig. 4. Molecular mechanism underlying c-Jun-mediated vascular smooth muscle cell proliferation.

including cell proliferation, transformation, differentiation, and apoptosis, depending on the cell type and the context of other regulatory influences that the cell is receiving. We have previously reported that AP-1 binding activity, composed of c-Jun, is significantly enhanced in injured rat artery by balloon angioplasty (Fig. 3A) (7) and angiotensin II-mediated hypertension (9), suggesting that c-Jun-related AP-1 may play some role in various vascular diseases. Therefore, we have examined the role of c-Jun-related AP-1 in vascular remodeling by using the gene transfer technique. To specifically inhibit AP-1 promotor activity, we have made a dominant negative mutant of c-Jun lacking the transactivation domain of wild c-Jun (DN-c-Jun) (Fig. 3B). In vivo transfection of DN-c-Jun significantly inhibits vascular smooth muscle cell (SMC) proliferation in the intima and the media after balloon injury and subsequently prevents intimal thickening (Fig. 3C) (12). Thus, AP-1 seems to be a potential therapeutic target for vascular remodeling. Furthermore, we have investigated the molecular mechanism underlying c-Jun-mediated vascular smooth muscle cell proliferation, by recombinant adenoviral infection containing DN-c-Jun. c-Jun contributes to the platelet-derived growth factor (PDGF)-BB- or serum-induced vascular SMCs proliferation through downregulation of $p27^{Kip1}$, activation of Cdk2, and the subsequent induction of $G_1/S$ transition (13) (Fig. 4).

## Conclusions and future directions

In a series of our recent work, we propose that three MAP kinases, including ERK, JNK and p38, and AP-1, even in transient activation, are all involved in the development of vascular remodeling, and therefore seem to be the promising therapeutic targets for restenosis after percutaneous transluminal coronary angioplasty. However, the technique of in vivo gene transfer has a clinical limitation because isolation of the arterial segment for gene transfer requires a longer period of time than that acceptable in the clinical situation. Therefore, the development of a more effective gene transfer technique is essential for the clinical application of these dominant negative MAP kinases and c-Jun. Furthermore, as arterial restenosis in humans after balloon injury generally occurs at longer periods than in rats, the development of novel vectors taking advantage of much longer duration of gene expression than the conventional vectors is important for clinical application. Thus, further study is needed to determine whether dominant negative MAP kinases and c-Jun can be applied to human coronary restenosis. The development of specific and potent pharmacological inhibitors of MAP kinases or AP-1 that can be used in vivo is also useful for as a new therapeutic strategy of vascular remodeling.

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